REMARKS

Claims 1, 4-8, 11-16, 20-25 and 34-40 are currently pending. Claims 1, 4-8, 11-16, 20-25 and 34 have been amended to clarify the application subject matter and to correct informalities. The amendments to the claims are further supported throughout the specification, for example in paragraphs [0038], [0097], and [0103] of the application publication (US 2008/0277080 A1) in original claim 19. New claims 35-40 have been added and are supported by original claims 1-25 and 34. The non-elected claims 2 and 26-33, along with claims 2, 3, 9, 10 and 17-19 have been canceled without prejudice. No new matter has been added.

In view of the foregoing amendments and the following remarks, Applicant respectfully submits that the claims are allowable and the case is in condition for allowance.

Compliance with 35 U.S.C. § 101 and § 112

Claim 21 was rejected under 35 U.S.C. § 101 for allegedly being drawn to nonstatutory subject matter. Claim 21 has been amended, per the Examiner's suggestion on page 3 of the Office Action mailed on October 8, 2010 ("the Office Action"), therefore it is respectfully submitted that claim 21 fully complies with 35 U.S.C. § 101 and the rejection should be withdrawn.

Claim 7, 20 and 25 were rejected under 35 U.S.C. § 101 and § 112, second paragraph for failing to recite a step. Claims 7, 20 and 25 have been sufficiently amended to obviate the rejections, thus all of the claims fully compliy with 35 U.S.C. § 101 and § 112, and the rejections should be withdrawn.

Claim 12 was rejected under 35 U.S.C. § 112 as allegedly being indefinite for failing to particularly point out and distinctly claims the subject matter regarded as the invention. Claim

12 has been amended to obviate the rejection, the claims fully comply with 35 U.S.C. § 112 and the rejection should be withdrawn.

Novelty under 35 U.S.C. § 102

Claims 1, 2 and 4-7 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Kobayashi (2002 Plant Cell Physiology 43: 1259-1265). Applicant respectfully disagrees.

However, in an effort to expedite prosecution, independent claim 1 has been amended and now recites,

> A gene expression cassette comprising one or more genes encoding UDP-D-glucuronate carboxylase (EC: 4.1.1.35), which is cloned into the transformation binary vector and introduced into the bacterium *Agrobacterium tumefaciens*, wherein the cassette is for expression in *Eucalyptus* cells.

Kobayashi simply fails to disclose (either expressly or inherently) the above claimed gene expression cassette. In contrast, Kobayashi is directed to the cloning and introduction of a cassette in *E. coli*. This is a clear distinction from the claimed subject matter directed to a gene expression cassette that is introduced into *Agrobacterium tumefaciens*, for expression in *Eucalyptus*.

Accordingly, it is respectfully submitted that claim 1 is allowable. Furthermore, claims 4-8, 11-16, 20-25 and 34-40, depend from and further define the subject matter of claim 1 and therefore are also allowable.

Patentability under 35 U.S.C. § 103(a)

Claims 1-25 and 34 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kobayashi in view of Cameron-Mills (US 6,031,155). Applicant respectfully disagrees.

However, as discussed above, in an effort to expedite prosecution, claim 1 has been amended, and now recites,

> A gene expression cassette comprising one or more genes encoding UDP-D-glucuronate carboxylase (EC: 4.1.1.35), which is cloned into the transformation binary vector and introduced into the bacterium *Agrobacterium tumefaciens*, wherein the cassette is for expression in *Eucalyptus* cells.

For at least the reasons discussed above, Kobayashi does not teach or suggest the above configuration. Moreover, Cameron-Mills fails to cure the deficiencies of Kobayashi.

Cameron-Mills is directed to the gene sequence encoding of barley endoxylanase, (see abstract). More specifically, Cameron-Mills is directed to a genomic nucleic acid sequence and the 62 kDa barley endoxylanase it encodes, which is used to express enhanced amounts of endoxylanase in host cells, particularly in plants transformed with the gene, permitting enhanced degradation of cell wall xylan (see, column 1, lines 5-8). Cameron-Mills fails to teach or suggest gene encoding of the claimed enzyme and/or *Eucalyptus* transformation by *Agrobacterium*. Cameron-Mills is directed to solve a specific problem related to the fermentation of barley, which is clearly different form the claimed subject matter. Cameron-Mills teaches the expression of a specific enzyme (endoxylanase) in a specific plant (barley), to solve different problems than the claimed subject matter.

Furthermore, although Cameron-Mills refers to the use of a sequence to transform host cells, the transformation method used is NOT *Agrobacterium* as recited in the present claims. Instead, Cameron-Mills describes several **disadvantages** in using *Agrobacterium* for

transforming the plants of interest (see, column 10, lines 49-53). Thus, Cameron-Mills *teaches away* from the claimed subject matter. Therefore, a person skilled in the art would not have found the claimed subject matter directed to *Eucalyptus* plant obvious based on the teachings of Kobayashi and Cameron-Mills.

In addition, Cameron-Mills is related to cell degrading enzymes, whereas the claimed subject matter recites an enzyme involved in a synthesis pathway. Furthermore, Cameron-Mills is related to the transformation of cereal plants in view of enhanced brewing processes. The claimed subject matter is related to *Eucalyptus* plants which can be used to improve wood and/or cellulose production.

Thus, considering the teachings of Kobayashi and Cameron-Mills together, a person skilled in the art would not have found it obvious to achieve the claimed *Agrobacterium* gene cassette or method based on Cameron-Mills because, as discussed above, Cameron-Mills teaches away from the claimed subject matter. Also, none of Kobayashi or Cameron-Mills discloses or suggests *Eucalyptus* transformation.

Importantly, although Kobayashi describes the UDP-D-glucoronate carboxylase gene, and Cameron-Mills discloses a method for transforming plants, a person having ordinary skill in the art would not find it obvious to combine the two references to achieve the claimed subject matter because, as noted in the below discussed references, achieving plant transformation is known to be extremely difficult.

Christensen JH *et al.* (2001, Molecular Breeding of Woody Plants, pages 171-176) – discusses the transformation of plants with constructs containing the cDNA of peroxidase cDNA PXP 3-4. Given the known role of peroxidases in lignin polymerization, overexpressing the enzyme through plant transformation was expected to produce alterations in the lignin amount of

the transformed plant, or even in its metabolic profile. However, it was observed that the transgenic plants were indistinguishable from the wild-type plants (see page 175, second paragraph), and no alteration was observed in the lignin amount, condensation or monoglinol compostion and the metabolic profiles and the redox state of these plants were unaltered (see page 171, abstract section).

Christensen JH *et al.* also states that the transformed plants showed unwanted side effects due to the peroxidase oxidase reaction (see page 175, second paragraph), corroborating the idea that simply having knowledge of the functions of a determined protein does not guarantee that a plant transformed to express said protein would present a desired phenotype without any disadvantage.

In addition, Burton RA *et al.* (2001, Molecular Breeding of Woody Plants, pages 77-84) explains that the plant transformation can be accompanied by genetic rearrangements that could interfere with expression patterns of unrelated genes or that could indirectly silence expression of the target structural gene through changes in genes encoding transcription factors (see page 82, second paragraph). In other words, due to the recognized unpredictability of the instant technology, a person having ordinary skill in the art would not have found the claimed subject matter obvious in view of the cited references.

Moreover, a person having ordinary skill in the art would not have found the claimed subject matter obvious in view of the cited references, as the claimed subject matter achieves significantly improved results that would not have been expected to a person having ordinary skill in the art based on the cited references, as explained below.

In order to demonstrate the effectiveness of the genetic transformation described in the present application, Applicant evaluated the monosaccharide and lignin content of three *Eucalyptus grandis* transformed plants.

The plants were transformed according to the methods described in the present application, with a gene expression cassette comprising the UDP-D-Glucoronate Carboxylase gene (UXS) from pea plants, via *Agrobacterium tumefaciens* transformation. The monosaccharide content of the cell wall was measured after sulfuric acid hydrolysis.

As shown in Table 1 below, the transformed plants (UXS 1, UXS 2 and UXS 7) were compared with non transgenic control plants (WT), and the analysis was performed with the following monosaccharides: fucose, galactose, glucose, mannose, arabinose, xylose, hexoses, pentoses and total monosaccharide content.

The results show a significant increase in the monosaccharide content, especially hexoses and pentoses of the transformed plants (UXS 1 and UXS 2) when compared to the WT plants. Table 3 below, shows the relative change, expressed as % of control line (mg/g dry weight).

Table 2 below, shows the pleiotrophic effect observed in the transformed plants in relation to the lignin content of the cell wall, which was decreased in the transformed plants, especially the insoluble fraction. In Table 4 below, it is observed the relative changes in the lignin content of the cell as a percentage, % of control line (mg/g dry weight).

Taken together, these data demonstrate that the genetic transformation of *Eucalyptus* plants with the UXS gene through *Agrobacterium* transformation was successfully and unexpectedly achieved, resulting in a change of the hemicellulose content of the wood.

Table 1 - Chemical composition of the cell wall (mg/g dry weight)									
Lines	**Fucose**	**Galactose**	**Glucose**	**Mannose**	**Arabinose**	**Xylose**	**Hexoses**	**Pentoses**	**Total**
WT	0,34	16,12	456,76	9,45	5,52	252,45	482,67	257,97	740,64
Uxs1	0,38	15,52	494,32***	10,73	6,26***	280,98***	520,95***	287,24***	808,19***
Uxs2	0,36	18,34	496,35***	10,94	5,98	268,72	525,99***	274,70**	800,69***
Uxs7	0,32	18,23	437,74	8,74	5,03	249,70	465,03	254,73	719,76
*Dunnet's test: **P<0.05, ***P<0.01*									

Table 2 - Lignin Content (mg/g dry weight)			
Lines	Insoluble Lignin	Soluble Lignin	Total Lignin
WT	241,72	35,19	276,91
Uxs1	229,67***	34,11	263,78***
Uxs2	227,50***	33,47	260,97***
Uxs7	228,36***	32,92	261,29***
*Dunnet's test: **P<0.05, ***P<0.01*			

Table 3 - Relative changes in monosaccharide content of the cell wall after sulfuric acid hydrolysis. Values are expressed as % of control line (mg/g dry weight).									
Lines	**Fucose**	**Galactose**	**Glucose**	**Mannose**	**Arabinose**	**Xylose**	**Hexoses**	**Pentoses**	**Total**
Uxs1	11.8	-3.7	8.2***	13.5	13.4***	11.3***	7.9***	11.3***	9.1***
Uxs2	5.9	13.8	8.7***	15.8	8.3	6.4	9.0***	6.5**	8.1***
Uxs7	-5.9	13.1	-4.2	-7.5	-8.9	-1.1	-3.7	-1.3	-2.8
*Dunnet's test: **P<0.05, ***P<0.01*									

Table 4 - Relative changes in Lignin Content of the cell wall after sulfuric acid hydrolysis. Values are expresses as a % of control line (mg/g dry weight)			
Lines	Insoluble Lignin	Soluble Lignin	Total Lignin
Uxs1	(-) 5.0***	(-) 3.1	(-) 4.7***
Uxs2	(-) 5.9***	(-) 4.9	(-) 5.8***
Uxs7	(-) 5.5***	(-) 6.5	(-) 5.6***
*Dunnet's test: **P<0.05, ***P<0.01*			

Accordingly, because none of the cited references, either taken alone or in combination, teach or suggest the claimed subject matter and because the claimed subject matter achieves unexpected results that would not have been obvious to a person having ordinary skill in the art, it is respectfully submitted that claim 1 is allowable over the cited references. Furthermore, claims 4-8, 11-16, 20-25 and 34-40, depend from and further define the subject matter of claim 1 and therefore are also allowable.

In view of the foregoing amendments and comments, Applicant respectfully submits that this application should be allowed and the case passed to issue. If there are any questions regarding this application, a telephone call to the undersigned would be appreciated.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

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